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IS 13933 (1995): Method of test for ready biodegradability of surface active agents (Modified sturm test) [CHD 25: Soaps and other Surface Active Agents]

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सतह सक्रिय अभिकर्मक तैयार बायोविकर्गीकरण की परीक्षण पद्धति (आशोधित स्टर्म परीक्षण)

Indian Standard

METHOD OF TEST FOR READY BIODEGRADABILITY OF SURFACE ACTIVE AGENTS (MODIFIED STURM TEST)

UDC 661·185·1 : 543

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

November 1995

Price Group 4

Soaps and Other Surface Active Agents Sectional Committee, CHD 025

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Soaps and Other Surface Active Agents Sectional Committee had been approved by the Chemical Division Council.

It was considered to incorporate the method of test for ready biodegradability of surface active agents (Modified Sturm Test) in IS 12795 : 1989 'Specification for Linear Alkyl Benzene'. However, the Committee decided to prepare a separate Indian Standard, prescribing the complete details of the test method which would be of special significance in the context of all soaps and surface active agents. The existing method in IS 12795 : 1989 provides for primary biodegradability. This standard provides a method to estimate the ultimate biodegradability which is more relevant in the context of environment protection.

The test method presently prescribed in IS 12795 : 1989 is applicable to soluble and insoluble organic materials. Since presence of organic impurities complicates the interpretation of the CO_2 production data, it has been provided that the material tested must be of high purity. In addition, it is subject to the following conditions at the concentrations used in test:

i) The test material should have negligible vapour pressure,

ii) It should not be inhibitry to bacteria,

iii) It should not significantly get adsorbed to glass surface, and

iv) The test has been found suitable for determining the ready biodegradability of organic chemicals under aerobic conditions.

In the preparation of this standard considerable assistance has been drawn from the Organisation for Economic Cooperation and Development (OECD) Doc ; 301B 'Method for determination of biodegradability of surface active agents' which is thankfully acknowledged.

The composition of the Technical Committee responsible for formulation of the standard is given in Annex A.

In reporting the results of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'.

Indian Standard

METHOD OF TEST FOR READY BIODEGRADABILITY OF SURFACE ACTIVE AGENTS (MODIFIED STURM TEST)

1 SCOPE

1.1 This standard prescribes the method of test for ready biodegradability of Surface Active Agents.

1.2 Outline of the Method

In this test, test organisms are inoculated into a chemically defined microbial growth medium containing the test material. Carbon dioxide-free air is bubbled through the contents and the amount of carbon dioxide produced is estimated by its absorption in barium hydroxide solution. The biodegradability is judged by the amount of carbon dioxide generated by the test material as a percentage of the theoretical amount that would be produced by the material.

1.3 Principle

Aerobic micro-organisms utilising an organic substrate as a carbon and energy source convert the molecule into new cells, carbon dioxide and water. Further, when the available substrate is exhausted, some bacteria die and are subsequently metabolised by the surviving cells. By measuring the amount of carbon dioxide produced and comparing the quantity with the theoretical yield, calculated from the substrate carbon content, a measure of the ultimate biodegradability can be made. The test duration is limited to 28 days.

NOTE — It has been found by experience that materials degrading extensively in this period are readily degraded under practical conditions. Hence, a result regarded as positive by this test procedure indicates that the substance tested is readily and ultimately biodegradable.

2 TOTAL ORGANIC CARBON OF TEST MATERIAL

2.1 The total organic carbon (TOC) content of the test material should be calculated or, if this is not possible, actually analysed as per details given in **2.2** to compute the theoretical yield of carbon dioxide.

2.2 Determination of Total Organic Carbon

2.2.1 *General*

This method, with suitable adjustment of the method of heating the sample depending on its composition, is useful in determining carbon and hydrogen in many organic compounds. Gas-heated furnaces may be used, but electrically heated ones are more easily manipulated.

2.2.2 *Apparatus*

The furnace of the Heraeus type consists of three independent heaters *A*, *B*, and *C* in Fig. 1. It is substantially the same as all furnaces for determining hydrogen and carbon by combustion. Two of these heaters, *A* and *B* connected to rheostat *F* and *G* are movable along the tube. The third, *C* which surrounds the lead chromate, is stationary. The form of furnace though not essential is highly convenient. The usual furnaces are not long enough, and the lead chromate has to be heated by a gas flame, with less flexibility and with frequent damage by melting to the tube.

As combustion tube, use a hard glass or fused silica with a transparent section the total length is 100 to 110 cm, and the external diameter is about 21 mm. Insert a rubber stopper at one end, and draw out the other sufficiently to make a rubber tubing connection to the absorption train. To allow observation the 12-cm transparent section should begin about 35 cm from the large end. The first part of the tube up to the end of the transparent section is empty. Following this space is a roll of copper gauze about 2 cm long; and next 33 cm is filled loosely with copper oxide in short lengths. A second roll of copper gauze separates the wire from 18 cm of fused lead chromate, which is held in place by a plug of glass wool extending to the constricted end of the tube.

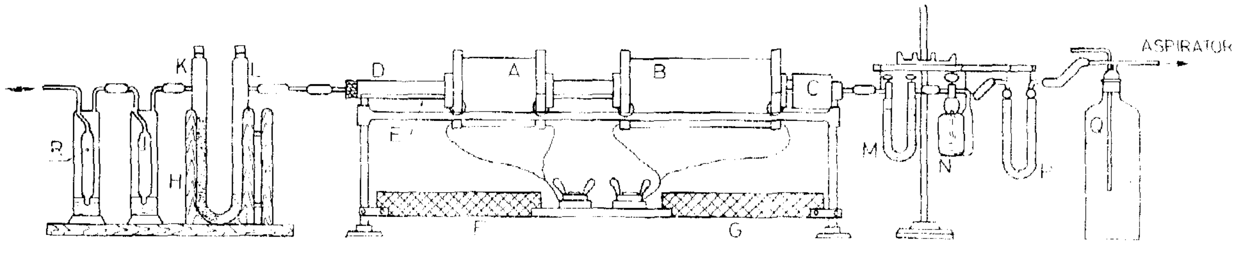

FIG. 1 APPARATUS FOR DETERMINATION OF TOTAL ORGANIC CARBON

Purify the oxygen or air entering the combustion tube by passing through a drying apparatus *H*, containing the following, arranged in order of the passage of the gas through them; sulphuric acid *R* for removing traces of ammonia, 30 percent potassium hydroxide solution *J*, granular soda lime *K* for removing traces of carbn dioxide, granular calcium chloride *L* for removing moisture. The absorption tube *R* is connected to an oxygen tank with a suitable reducing valve.

The absorption train consists of a 5-inch tube *N* filled with granular calcium chloride to absorb moisture. Saturate the calcium chloride, before use with carbon dioxide to prevent possible absorption of the later from the sample by any traces of calcium oxide that may be present. This is conveniently done by passing carbon dioxide through a tower filled with calcium chloride or by filling a large jar containing calcium chloride with carbon dioxide and then allowing it to stand overnight. In either case, finally draw air through to remove any carbon dioxide. Keep the prepared calcium chloride in well-stoppered bottles.

Connect the calcium chloride tube to a potash bulb *N* containing a 30 percent potassium hydroxide solution and some soda lime and granular calcium chloride. Use a little potassium permanganate to oxidize an ferrous iron or nitrates in the potassium hydroxide solution. Normally, six to eight determinations may be made with one charge in the bulb. The Vanier tube shown may be replaced by the more common Geissler tube or, better, two in series. Follow these by a soda lime-calcium chloride tube *P*. Connect the last tube to an aspirator, using a guard tube *Q* to keep the pressure constant. The soda lime and granular calcium chloride to absorb any carbon dioxide escaping from the potassium hydroxide solution and any water evaporating from it.

2.2.3 *Procedure*

Assemble the apparatus, and test for tightness by starting the respiration of air at the rate of about three bubbles per second through the potassium hydroxide bulb; then close the inlet for air or oxygen at the opposite end of the train. If there are no more than three bubbles per minute in the potash bulb, the apparatus is considered tight.

Before starting the determination, after the train has been out of use some hours or after any changes in chemicals and connections, run a blank by aspirating about a litre of air through the train, which is heated in the same manner as if a determination were being made. If the Vanier bulb or Geissler tubes and the tube containing calcium chloride show a change in weight of less than 0.5 mg each, the apparatus is ready for use.

Starting from the cold, move the heaters towards the end of the tube where the gases enter, and warm the air enough by cutting in the electric current to dry the tube and its contents thoroughly. Cut off the current from the small heater *A* and move the large heater *B* over the copper oxide. Keep about 250 mm of that part of the combustion tube between the two heaters exposed where the boat containing the sample is to be placed. Bring the copper oxide to a red heat by turning on the full current on the large heater. When this temperature is reached, reduce the current with the rheostat to avoid overheating the tube. This is important, for a fused tube means much wasted work.

Meanwhile, weigh and connect the absorption train. It is advisable to use a bone-dry sample in this determination; otherwise, the moisture must be determined on a similar sample and the weight later subtracted from the increase in weight of the calcium chloride tube before the percentage of hydrogen is calculated. Weight 0.2 g of the finely meshed coal sample into a porcelain or platinum boat. Place the boat and sample in a weighing tube closed with a stopper to prevent moisture absorption until it is introduced into the combustion tube. When ready, place the boat and sample as rapidly as possible in the exposed and cooler part of the tube between the two heaters. The copper oxide should be red-hot and the lead chromate at dull red heat at this stage. As soon as the boat is in place near the asbestos plug at the beginning of the copper oxide, insert the stopper connecting with the purifying train and aspirate pure oxygen gas through the apparatus at the rate of about three bubbles per second.

Pass the current through the shorter heater. Control the rate of evolution of volatile matter by manipulation the rheostat and by gradually pushing this heater toward the boat. The heat must be increased gradually until all the volatile matter is driven off. Prevent too rapid and evolution of gas and tar, which would thus escape complete combustion or be driven back into the purifying train. After the sample is ignited, increase the heat more rapidly but take care not to overheat and soften the combustion tube.

Continue the aspiration with oxygen for 2 minutes after the sample ceases to glow. Then turn off the current, and aspirate about 1 200 ml of air through the apparatus. Disconnect the absorption bulbs, cool to room temperature, wipe with a lintless cloth and weigh. Weigh the ash in the boat, and carefully inspect for any unburnt carbon, which would vitiate the results. The percentage of ash found should be checked with that determined by proximate analysis.

2.2.4 *Calculations*

$$\text{Increase in weight of potassium hydroxide bulbs} \times 0.272\,9 \times \frac{100}{\text{weight of sample}}$$

$$= \text{percent carbon}$$

3 DETERMINATION OF BIODEGRADABILITY

3.1 Apparatus

Assembly as shown in Fig. 2 consisting of the following:

3.1.1 *Corbon Dioxide Scrubbing Apparatus*

Four one-litre plastic bottles or glass flasks each containing 700 ml of 10 N sodium hydroxide (NaOH) solution.

One one-litre Erlenmeyer flask containing 700 ml of 0.025 N barium hydroxide [Ba $(OH)_2$]solution.

One empty one-litre Erlenmeyer flask as a trap to prevent liquid carry-over.

All these are connected in series, using suitable plastic tubing, to a pressurized air source, the air is sparged through the scrubbing solutions at a constant rate of flow.

3.1.2 *Carbon Dioxide Production Apparatus*

Four 5-litre flasks for each test material.

Bubblers (100-ml barium hydroxide absorbing solutions) —18 numbers.

Stoppers and flexible plastic tubings.

4 REAGENTS

4.1 Purity of Water

Sterilised distilled or deionised water free of compounds inhibitory to microbial survival must be used.

4.2 Mineral Salts Medium

The medium shall be prepared from the following stock solutions.

4.2.1 *Ferric Chloride Solution*

Dissolve 0.25 g ferric chloride hexahydrate ($FeCl_3$. $6H_2O$) in one litre of water.

4.2.2 *Magnesium Sulphate Solution*

Dissolve 22.5 g of magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) in one litre of water.

4.2.3 *Calcium Chloride Solution*

Dissolve 27.5 g of anhydrous calcium chloride in one litre of water.

4.2.4 *Phosphate Buffer Solution*

Dissolve 8.5 g of potassium dihydrogen orthophosphate (KH_2PO_4), 21.75 g of di-potassium hydrogen orthophosphate (K_2HPO_4), 33.4 g of disodium

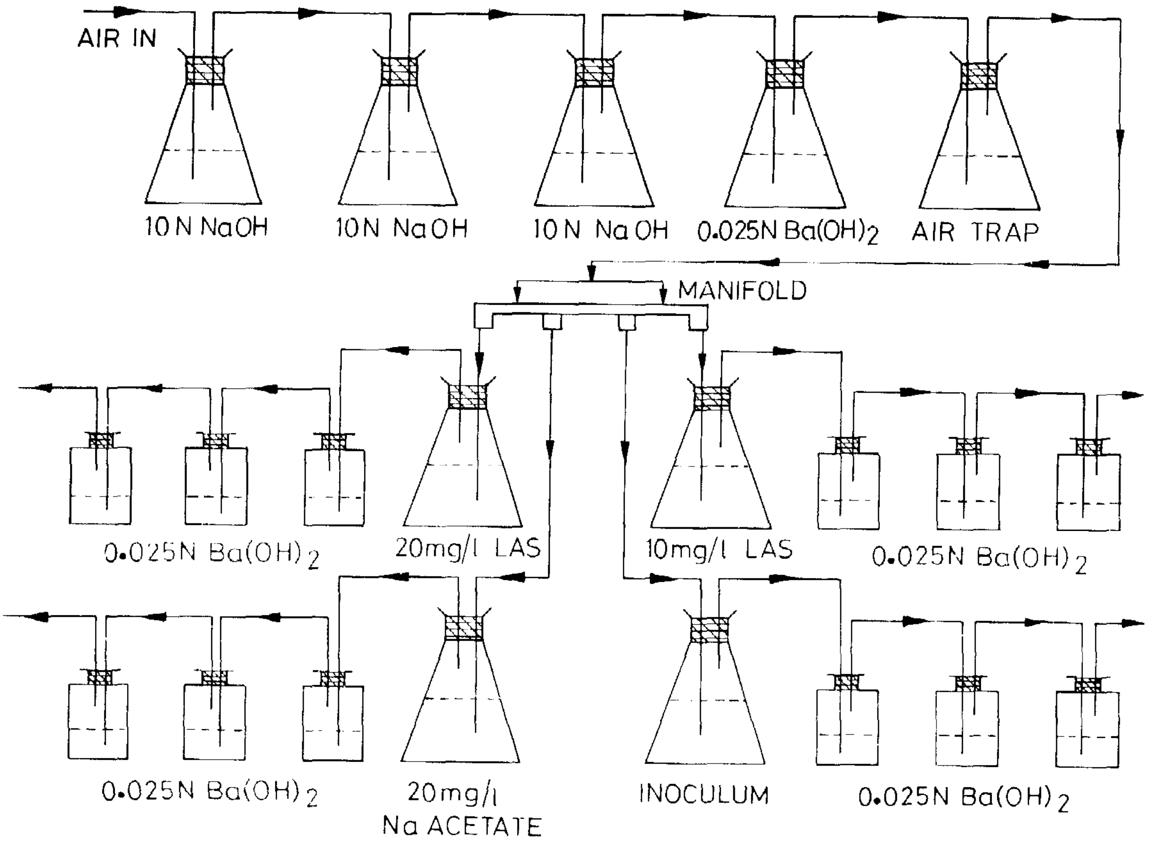

FIG. 2 OECD 'MODIFIED STRUM TEST' FOR READY BIODEGRADABILITY

hydrogen orthophosphate heptahydrate ($Na_2HPO_4.7H_2O$) and 1.7 g of ammonium chloride (NH_4Cl) in one litre of water.

4.2.5 *Ammonium Sulphate Solution*

Dissolve 40 g of ammonium sulphate $(NH_4)_2SO_4$ in one litre of distilled water.

4.3 Test Medium

Make up to one litre with water the following volumes of stock solution (**4.2**):

4 ml of ferric chloride solution,

1 ml of magnesium sulphate solution,

1 ml of calcium chloride solution,

2 ml of phosphate buffer solution, and

1 ml of ammonium sulphate solution.

The final solution will constitute the test medium.

4.4 Barium Hydroxide Solution — 0.025 N.

Dissolve 4.0 g of barium hydroxide octahydrate $Ba(OH)_2.8H_2O$ in one litre of water. Filter through a filter paper and seal the clear solution to protect from air and carbon dioxide. When running a series, it is preferable to prepare more than 5 litres of solution.

4.5 Hydrochloric Acid Solution — 0.5 N.

4.6 Test Organisms

4.6.1 *Source*

The source of the test organisms is activated sludge freshly sampled from a well-operated municipal sewage treatment plant receiving no or minimal industrial effluent.

4.6.2 *Preparation of Microbial Culture*

The activated sludge is aerated for 4 hours in the laboratory 500 ml of the mixed liquor is sampled and homogenised for 2 minutes at medium speed in a blender. It is then settled for 30 minutes.

If the supernatant still contains high levels of sludge solids at the end of 30 minutes, it may be settled for an additional 30 to 60 minutes or adapted to laboratory conditions to obtain better settling.

The supernatant is decanted to provide sufficient volume for 1 percent inoculum for each carbon dioxide test flask. Carry-over of sludge solids should be avoided, otherwise this would interfere with measurement of carbon dioxide produced.

4.6.3 *Measurement of Viable Count*

It is useful to perform viable counts on the supernatant fraction to determine microbial numbers; this is optional. The inoculum should normally contain 10^6 to 20 x 10^6 colony forming units per millilitre. It should be used the same day it was prepared.

5 PROCEDURE

5.1 Preparation of Sample

Prepare a stock solution of sample by dissolving a homogeneous aliquot of the test material in water, the concentration would depend on the active content percentage. To obtain a homogeneous sample, it is necessary to mix well, at the same time avoiding forming which will tend to concentrate the active ingredient disproportionately. This step is extremely important for correct calculation of biodegradation based on the exact amount of carbon in test system.

The *p*H of the stock solution need not be adjusted unless it falls outside the range of 3 to 10, since the phosphate buffer in the test medium will control it. If the *p*H lies outside this range, adjust an aliquot of the stock solution to *p*H 7.0 + 1.0 with 1N hydrochloric acid or sodium hydroxide making sure that the solution is vigorously mixed during the additon of acid or alkali.

5.2 TOC of Test Compound

Determine the total organic carbon (TOC) of the test material by analysing the stock solution (or the neutralised aliquot).

5.3 Reference Substances

In order to check the activity of the inoculum, the use of control substances is desirable. Aniline, sodium acetate or sodium benzoate are suggested as reference substances. The reference substance shall yield more than 60 percent dioxide within 28 days, otherwise the test is regarded as invalid and must be repeated an inoculum from a different source.

5.4 Test Conditions

The following steps shall be done in sequence:

To each of the four 5-litre test flasks, add 2 470 ml of water followed by 3 ml of ammonium sulphate, 3 ml of magnesium sulphate, 3 ml of calcium chloride, 6 ml of phosphate buffer, 12 ml of ferric chloride (all stock solutions), and 30 ml of activated sludge inoculum. These additions now total 2 527 ml in each flask.

Aerate the mixtures with carbon dioxide-free air (**3.1.1**) for 24 hours to purge the system of carbon dioxide.

After the aeration period, three carbon dioxide absorber bottles are each filled with 100 ml of 0.25 N barium hydroxide solution and connected in series to the exit air line of each test flask.

Now, test material is added to two of the four flasks to begin with testing period. Each material is tested at two concentrations, 10 and 20 mg/1. The amount of test material (stock solution) required in the flask is calculated as given below:

$$\text{Stock solution per flask, ml} = \frac{B \times C}{A}$$

where

A = test compound concentration in stock solution, mg/l;

B = test compound concentration in test flask, mg/l; and

C = final volume of test medium in the test flask (ml).

Sufficient stock solution to reach the desired test concentration, as calculated above, plus sufficient water to make 473 ml (stock solution + water) are added to the appropriate flasks. To the third flask, used as blank control and containing no test material, add 473 ml of water. The final volume in each flask is now 3 000 ml.

A control substance (**5.3**), at a concentration of 20 mg/1, is added to the fourth flask.

5.5 Performance of the Test

To start with, bubble carbon dioxide-free air (**3.1.1**) through the solutions at a rate of 50-100 ml/min per flask (approximately 1 to 2 bubbles per second). The carbon dioxide produced in each flask reacts with the barium hydroxide and precipitates as barium carbonate. The amount of carbon dioxide produced is determined by titrating the unreacted barium hydroxide with standard 0.05 N hydrochloric acid. Periodically, every 2 or 3 days, the carbon dioxide absorber nearest to the flask is removed for titration. The remaining two absorbers are moved closer to the flask, and a new absorber filled with 100 ml of fresh barium hydroxide solution is placed at the far end of the series. Titrations are made as needed (before any barium carbonate precipitate is evident in the second trap) approximately every alternate day for the first 10 days, and every fifth day until the 28th day.

On the 26th day, the *p*H of the flask contents is measured again, and then 1 ml of concentrated hydrochloric acid is added to each of the test flasks to decompose inorganic carbonate. The final titration is made on day 28.

Titrations of the 100 ml barium hydroxide solution are made after removing the bottles closest to the flask. The barium hydroxide is titrated with standard hydrochloric acid, using phenolphthalein indicator.

The test is run at 25 + 2° C temperature which is recorded throughout the duration of the test.

5.6 Reporting of the Results

The amount of carbon dioxide produced by the test compound during the test is measured and expressed as percent of the theoretical carbon dioxide it should have produced (TCO_2) as calculated from the carbon content of the test compound (TOC). Biodegradability is expressed as percentage TCO_2.

$$TCO_2 = \text{mg } CO_2 \text{ per mg of test material}$$

$$= \frac{(\text{number of carbon atoms in test material} \times \text{molecular weight of } CO_2)}{\text{molecular weight of active test material}}$$

Example: Sodium lauryl sulphate (SLS)contains 12 carbon atoms and therefore,

$$TCO_2 \text{ of SLS} = \frac{12 \times 44}{288.33} = 1.831 \text{ mg } CO_2/\text{mg SLS}$$

In the case of mixtures, TCO_2 of the total active material is weighed average of the TCO_2 of the individual compound.

5.7 Precaution

Evolution of a significant amount of carbon dioxide from the blank flask during test would indicate contamination or the medium, glassware or air supply. A total carbon dioxide evolution in the blank at the end of the test exceeding 50 mg carbon dioxide per 3 litres medium should be considered as invalidating the test.

5.8 Calculations

The first step in calculating the amount of carbon dioxide produced is to correct the test material flasks for endogenous carbon dioxide production. The control flask serves as a "seed blank" to correct carbon dioxide which may be produced through endogenous respiration of the bacteria. The amount of carbon dioxide produced by a test material is determined by the difference (in ml of titrant) between the experimental and blank barium hydroxide traps.

Sample Calculation:

Blank	48.0 ml	hydrochloric acid titrated
Experimental	45.0 ml	hydrochloric acid titrated
Test Material	3.0 ml	hydrochloric acid titrated

Then convert ml hydrochloric acid titrated into mg of carbon dioxide produced.

$$\text{Carbon dioxide, mg} = \frac{(0.05) \times \text{ml titrated}}{2} \times 44$$

$$= 1.1 \times \text{ml hydrochloric acid titrated}$$

The final step is calculating the theoretical carbon dioxide produced using the following formula :

$$TCO_2\text{, percent} = \frac{\text{mg } CO_2 \text{ produced}}{(\text{mg test material added}) \times (TCO_2 \text{ of test material})} \times 100$$

$$\text{Biodegradability, percent} = \frac{\text{Amount of } CO_2 \text{ produced}}{\text{Theoretical amount of } CO_2} \times 100$$

5.9 Interpretation of the Results

5.9.1 Test materials giving a result to greater than 60 percent yield of carbon dioxide (within 28 days) are regarded as readily biodegradable. This level must be reched within 10 days of biodegradation exceeding 10 percent.

ANNEX A
(*Foreword*)

COMMITTEE COMPOSITION

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(*Continued on page* 8)

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Amendments Issued Since Publication

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